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β-catenin Immunohistochemical expression in papillary thyroid cancer versus benign thyroid lesions in a sample of Iraqi patients

Eaman Suud Khalifa
Lecturer/pathology department/college of medicine/Mustansiriyah university/Baghdad. Iraq

Abstract
Background and objective: Papillary thyroid cancer is the commonest one among thyroid cancers. β -catenin is thought to be involved in thyroid cell to cell adhesion and proliferation. This study was aimed to determine β -catenin expression in benign and malignant tumors and its correlation to tumor differentiation.
Methods: Fifty three paraffin blocks of malignant and benign thyroid samples were collected from patients underwent thyroidectomy. Paraffin blocks were stained with Rabbit monoclonal to β -catenin.
Results: Most of papillary thyroid carcinomas subtypes are well differentiated (71%). Out of 15 cases of benign thyroid tumors, 11 cases showed a membranous staining (73.3%). Out of 38 cases of papillary thyroid carcinomas, 17 cases showed a membranous staining (44.7%). There was a significantly higher cytoplasmic staining in papillary thyroid carcinomas than benign lesions (39.5% vs 20%). There was no significant nuclear staining in benign lesions and papillary thyroid carcinomas.
The membranous staining was significantly lost in cases of poorly differentiated and undifferentiated papillary thyroid carcinomas.
Conclusion: β-catenin shifting from the membrane to the cytoplasm in papillary thyroid carcinoma is helpful in its differentiation from benign lesions.

Keywords: β-catenin, immunohistochemistry, papillary carcinoma, thyroid

Introduction

Thyroid cancer is the most frequent endocrine malignancies worldwide and comprises 1% of human malignancies. The most common thyroid cancer is papillary carcinoma (PTC) and constitutes 75-85% of all thyroid tumors (1, 2). It is affect females more than males with mean patient age at time of presentation around 40 years (3).
The increasing incidence of thyroid cancer might be related to the early diagnosis of asymptomatic small carcinomas due to the widespread screening (4).
PTC recurrence occurs in 5-20% of cases and this is usually resulting from inappropriate management and or aggressive tumor behavior. The recurrence might be occurs 20 years after primary diagnosis (5). Ten to 15% of PTC might be presented with distant metastasis and eventually this will reduce 10 years survival to 40 % (6).
Histological examination is the classical method for the diagnosis of PTC and it depend mainly on the morphological changes like "nuclear clearing, overlapping, grooving, and pseudoinclusions". When these changes are absent, there are some difficulties in distinguishing follicular variant of PTC (FVPTC) from nodular thyroid lesion (adenoma) and also there is a difficulty in differentiation between PTC and hyperplastic papillary thyroid nodule. Additionally, inter-observer variability is noted between pathologists, which eventually result in inappropriate treatment.
There are numerous genetic mutations that have been found to affect the clinical course of PTC. BRAF mutation is an example, which can lead to an aggressive course of PTC in approximately two third of patients. RAS and RET mutations also have been observed to in the determining the PTC clinical course (7-9).
Although, many reports have studied immunohistochemical expression of different tumor biomarkers as a diagnostic method for thyroid cancer, still there are controversies regarding the usefulness of many markers (10).

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In the last few years, β -catenin role in different human cancers have been highlighted and its role in cancer cells have described in details (11). Additionally, its role in "propagation , renewal and regeneration of epithelial tumors" have been evaluated (12-15).
β-catenin gene mutations have been found in hepatocellular carcinomas and other types of malignancies like APC-wild-type colon cancers (16–18).
At first, β-catenin was described as a protein coupled with E-cadherin, it has a critical role in the regulation of cell-cell adhesion (19,20).
β-catenin role in transformation of thyrocyte to a malignant cell is related to the "Wnt signaling pathway". Normally, β-catenin level in the cytoplasm is preserved in low level.
However, when the level of β-catenin increased, it will shift to the nucleus and then activate the Wnt pathway and production of protein (21).
Thyroid tumors have different clinical courses, some of them treated surgically (thyroidectomy) with radioiodine therapy. Because of the disease recurrence, the patient may underwent multiple courses of surgical treatment and radioiodine therapy. β -catenin role in disease recurrence, clinical course and prognosis is still under evaluation.

Aim of the study

In this paper, we assessed β-catenin staining as a diagnostic method in both benign thyroid lesions and PTC.

Materials and Methods

In this retrospective study, we selected 53 samples with the diagnosis of PTC (38 cases) and benign thyroid tumor (15 cases) , from patients underwent thyroidectomy in the period between 2018 and 2021 , and obtained their paraffin blocks from Teaching laboratories of Baghdad medical city , Baghdad, Iraq.
All samples were rechecked by two expert pathologists to assert the diagnosis. American Joint Commission on Cancer 7th edition was used for classification of tumor stage and additional pathological findings

Immunohistochemistry

The staining was done by the use of paraffin block after cutting of 4 um thickness sections and application of 3% hydrogen peroxide to block the enzyme activity in the background. The antibody used here is Rabbit monoclonal antibody reactive with β –catenin**.** Antigen retrieval was done by the use of citrate buffer (PH 6.0).

Statistical Analysis

“Statistical significance was determined by Fisher’s and Chi-square analysis for categorical variables". "categorical data were described as frequency (%). Chi-square test was also used to analyse the difference between the indices. P value less than 0.05 was considered to be statistically significant”

Results

In 53 selected cases , there were forty four women patients and 9 men patients (male to female ratio was 1:5.9) with mean age of 44.16 ± 1.27 years (range: 23–72).
Twenty nine cases (76.3%) of PTC showed a classical subtype features. While 7 cases (18.4%) were follicular variant (FVPTC) and 2 cases (5.3%) were papillary microcarcinoma.
β-catenin immunostaining pattern in benign thyroid lesions and PTC is summarized in table 1.
Out of 15 cases of benign thyroid tumors, 11 cases showed a membranous staining (73.3%).
Out of 38 cases of PTC, 17 cases showed a membranous staining (44.7%).
There was a significantly higher cytoplasmic staining in PTC than benign lesions (three benign cases showed a cytoplasmic staining (20%) while 15 cases of PTC showed a cytoplasmic staining (39.5%)).
There was no significant nuclear staining in benign lesions and well differentiated PTC.
The correlation between β-catenin immunoreactivity and PTC differentiation is summarized in table 2
Out of 38 cases of PTC, 27 cases were well differentiated (71%) and the remaining was poorly differentiated (6 cases) and undifferentiated (5 cases).

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The membranous staining was significantly lost in cases of poorly differentiated and undifferentiated PTC with predominant cytoplasmic staining.
Out of 38 cases of PTC, 21 cases (55%) showed inclusion bodies.

Discussion

Histological examination using H and E staining is the gold standard method for the diagnosis of PTC. However, sometimes there is an overlapping morphological features between malignant and benign lesions. It is not uncommon that distinguishing follicular lesions from carcinomas being difficult especially in case of FVPTC and also distinguishing PTC from hyperplastic papillary thyroid nodule is usually difficult in many patients (24). Precise pathological diagnosis is usually resulting in appropriate treatment and eventually avoiding serious complications. Immunohistochemical staining has been emerging as an important alternative diagnostic tool for thyroid tumors (10) . Previous studies have concluded that increasing level of β-catenin in the cells is important factor in the development of thyroid cancer and other types of malignancies (19).
In previous literatures, there is a major role of β-catenin in cell adhesion and the Wnt signaling pathway. Wnt signaling pathway activation might be involved in the tumorigenesis of the thyroid cancer especially papillary and follicular carcinomas (19).
In adenocarcinoma and follicular carcinomas, there is a reduction in β-catenin membranous expression, and this reduction is associated with loss of differentiation (25).
There is a major role for Wnt/β -catenin in tumor initiation and expansion. Normally, β -catenin is found in cell to cell junctions that are bound to E cadherin (20-21). β -catenin is released after activation of Wnt pathway and then accumulates in cytoplasm. After that it enters the nucleus and affects genes that responsible for proliferation (e.g c-myc and cyclin D1) (26, 27).
Therefore, β -catenin pathway, E-cadherin bound part, have a major role in PTC clinical course and its progression. E-cadherin and β -catenin binding have a major role in cell to cell adhesion. The loss of E-cadherin and β -catenin binding is correlated to progression of tumor.
Sethi K et al, reported in there study that β -catenin was a reliable diagnostic marker for PTC (10). In Zhang et al. study, expression of β -catenin was higher in cases that have nodal metastasis than that in primary tumors, but this difference was statistically not significant (27).
In our study, 83% of patients were females (male to female ratio was 1:5.9)
The most frequent subtype of PTC was the classical subtype (76.3%) followed by FVPTC (18.4%) and papillary microcarcinoma (5.3%).
Benign thyroid lesions like follicular adenoma showed a strong membranous staining, weak cytoplasmic staining and marked loss of nuclear staining (Table-1) and this finding was similar to many previous reports.
There was a significant co-relation between the decreasing of memberanous β -catenin staining and progressive loss of tumor differentiation (p value <0.05) (Table-2).
Gracia-Rostan et al. reported in there study that carcinomas cases showed a significantly lower membranous β-catenin staining comparing to follicular adenomas. Additionally, they found that reduction of memberanous β -catenin was correlated to the progressive loss of tumor differentiation (35).
Membranous H-score was higher in well differentiated cases (199 ± 90) compared to poorly differentiated cases (114 ± 70). In cases of well differentiated carcinoma, H score was higher (168) than that in poorly differentiated cases (115). There was a significant decrease in the memberanous β -catenin H score in both subtype during the progression from well differentiated to a poorly differentiated tumor.
Rossi et al, found in there study that poorly differentiated PTC showed HBME-1 and Galectin-3 expression , additionally they observed that β -catenin expression was lost in undifferentiated PTC cases , in addition to that, it was linked to the vascular invasion and distant metastasis and eventually higher mortality and they concluded that β -catenin is important prognostic factor (28).
We observed in this report that the nuclear expression of β-catenin was not significant in both benign thyroid lesions and well differentiated PTC.
Regarding poorly and undifferentiated tumors, our study showed that there was a reduction or complete loss of membrane β -catenin staining with predominant cytoplasmic staining. Additionally, we observed that the

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nuclear staining was higher than that in well differentiated cases (p value<0.05) (Table-2) and therefore this indicate that the nuclear accumulation of β-catenin is a sign for tumor dedifferentiation.

Similarly , Miyake et al., observed in there study that there is a membranous and cytoplasmic localization of β-catenin in cases of well differentiated PTC, while in cases of undifferentiated and poorly differentiated carcinomas the accumulation of β-catenin was higher in the nucleus. They reported in there study that this was due to β-catenin coding gene mutation (22).

Membrane expression of β-catenin was seen in both benign and malignant lesions (73.3% and 44.7%, respectively). These findings were consistent with many previous studies.

Rezk et al., found in their study that β-catenin membrane expression in malignant thyroid tumors was 87% and in benign thyroid lesions was 79% (34).

In this study, 39.5% of PTC showed a cytoplasmic expression of β-catenin.

Garcia-Rostan et al. (35) reported in their study that the cytoplasmic expression of β-catenin in PTC was 100% increased. Similarly, Meirmanov et al. (36) observed the same result.

Sethi et al. (10) observed in their study that the immunohistochemical expression of β-catenin was 96% in PTC and 100% in benign lesions. Furthermore, He et al. (37) reported that β-catenin expression in PTC was 71.7%.

Out of 38 cases of PTC, 21 cases (55%) showed inclusion bodies and this was more evident in poorly and undifferentiated PTC cases.

In Resk et al. study (33) , 88% of the classical (conventional) PTC cases 20% of the FVPC cases showed a strongly positive staining of intranuclear pseudoinclusions and the staining was variable from case to case and was not related to the intensity of the cytoplasmic or nuclear β-catenin staining . This relation between β-catenin and pseudoinclusions give a suggestion that β-catenin may be involved in the development of these pseudoinclusions.

β-catenin is participate in organizing actin and polymerization of microtubule (38), when these particles released from a β-catenin focus and pushed on the nuclear envelope resulting in development of these inclusions.

Conclusion

We can conclude in this study that β-catenin shifting from the membrane to the cytoplasm in PTC is a helpful diagnostic tool in the differentiation between benign thyroid lesion and PTC. Additionally, there is a correlation between the loss of membranous β -catenin staining and the aggressive behavior of the tumor. β-catenin also can be used as an immunohistochemical marker for clarifying specific morphological changes such as intranuclear pseudoinclusion bodies, that sometimes be difficult to observed in conventional H and E staining.

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Conflicts of interest: No conflict of interest reported.

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Table 1: β-catenin expression in malignant and benign thyroid tumors

Tumor type	β-catenin expression		
	Membranous	Cytoplasmic	Nuclear
Benign (n=15)	11 (73.3%)	3 (20%)	1(0.6%)
PTC (n=38)	17 (44.7%)	15(39.5%)	6(15.8%)
Total (n=53)	28(52.8%)	18(34%)	7(13.2%)

Table 2: β-catenin immunoreactivity in relation to PTC differentiation

Differentiation	β-catenin expression			P value
	Membranous n (%)	Cytoplasmic n (%)	Nuclear n (%)	

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Well differentiated (n=27)	16 (59.2%)	8 (29.6%)	3 (0.11%)	
Poorly differentiated (n=6)	1 (16.6%)	4 (66.6%)	1 (16.6%)	P<0.05
Undifferentiated (n=5)	0	3 (60%)	2 (40%)	
Total (n=38)	17 (44.7%)	15 (39.5%)	6 (15.8%)	

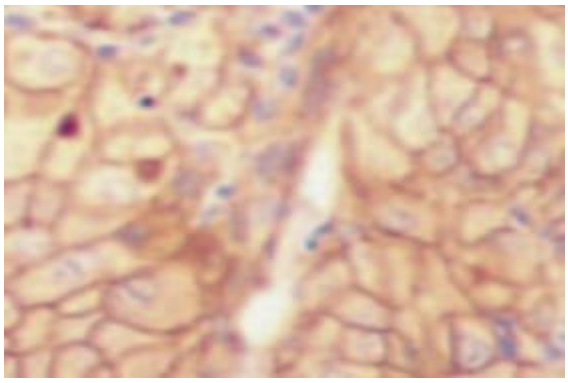

Figure 1: Positive membranous immunostaining for β-catenin

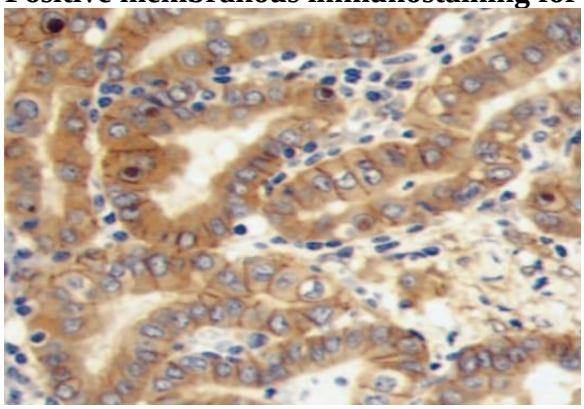

Figure 2: Positive cytoplasmic immunostaining for β-catenin with positive pseudoinclusion

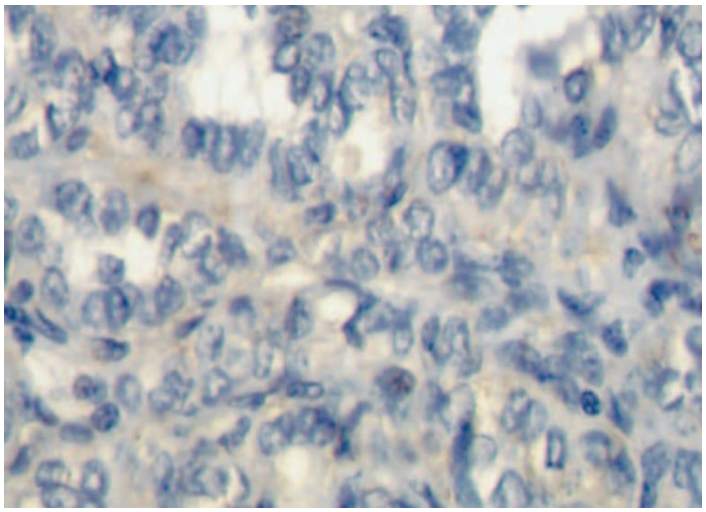

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Figure 3: Papillary thyroid carcinoma with negative immunostaining for β-catenin